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The Presence of at Least Two Different H-Blood-Group-Related β-D-*Gal* α-2-L-Fucosyltransferases in Human Serum and the Genetics of Blood Group H Substances

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SUMMARY

Sera from H normal, secretors and nonsecretors (*H/-*, *Se/-* and *H/-*, *se/se*), as well as from H-deficient secretors (*h/h*, *Se/-* or Bombay secretors) contain enzyme(s) for the transfer of L-fucose in the α-configuration to the 2-position of suitable β-D-galactopyranosyl units. Sera from H-deficient nonsecretors (*h/h*, *se/se*; i.e., Bombay nonsecretors) are devoid of such β-D-*Gal* α-2-L-fucosyltransferase(s). In order to study these enzymes, a comparison was made of the kinetic properties of the enzymes present in the sera of H-normal nonsecretors (*H/-*, *se/se*) with those of H-deficient secretors (*h/h*, *Se/-*). These studies revealed a clear difference between the two sources of enzyme: (1) the apparent K_m for GDP-fucose was four times lower with the H-normal nonsecretor serum (0.008 mM) than with the H-deficient secretor serum (0.028 mM); (2) acceptors with a type 1 or type 3 chain proved to be better than acceptors with a type 2 chain or than phenyl-β-D-galactopyranoside for the enzyme present in the serum of H-deficient secretor individuals. Indeed, the synthetic type 2 compound, βDGal (1→4)-3-deoxy-β-DGlcNAc-l-OCH_3, which cannot act as an acceptor of β*DGlcNAc* α-¾-L-fucosyltransferases, remained unchanged in the serum of an H-deficient secretor but was a good acceptor in the serum of an H-normal nonsecretor, and (3) the α-2-L-fucosyltransferease activity of the H-deficient secretor serum was more sensitive to heat inactivation than that of the H-normal nonsecretor serum (t½ at 46°C were 10 min and 75 min, respectively). These results show that at least two distinct α-2-L-fucosyltransferases are present in human serum. It is concluded that the enzymatic activity

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found in the H-deficient secretor serum (*h/h*, *Se/-*) could be the product of the *Se* gene and the enzymatic activity found in the H-normal nonsecretor serum (*H/-*, *se/se*) could be the product of the *H* gene. This conclusion correlates well with the finding that *H* and *Se* genes are closely linked and might have derived by gene duplication in the course of evolution.

INTRODUCTION

The minimum structure for the H antigenic determinant is a terminal αLFuc)1→2)DGal unit, which is the biochemical precursor of the A and B blood group determinants. Structural substitutions by *N*-acetylgalactosamine and galactose give, respectively, the A (αDGalNAc(1→3) [αLFuc(1→2)]βDGal), and the B (αDGal(1→3)[αLFuc(1→2)]βDGal) determinants. Three main types of precursor chains are known: the type 1 chain [βDGal(1→3)βDGlcNAc-R], the type 2 chain [βDGal(1→4)βDGlcNAc-R], and the type 3 chain [βDGal(1→3)DGalNAc], and, accordingly, the ABH determinants are referred to as types 1, 2, and 3. These antigens are widely distributed in tissues, and erythrocytes are among the cells that express ABH antigens. The H antigen is readily found in the saliva of about 80% of normal individuals, and these are referred to as ABH secretors. The remaining normal individuals are referred to as ABH nonsecretors. However, there exists a rare group of individuals who lack the precursor H substances on the red cells (H-deficient phenotype) and, consequently, are also A and B deficient. Nevertheless, among these persons having H-deficient red cells, some are classified as secretors since these have normal amounts of H antigen in saliva. Thus, four classes of individuals are identified: people with a normal expression of H on red cells but who may or may not secrete ABH substances in saliva and people who possess H-deficient red cells but, similarly, may or may not secrete ABH substances into their saliva.

A classical genetic model has been proposed in order to explain these four phenotypes [1]. In this model, a dimorphic locus *H-h* has a dominant allele *H* coding for an α-2-L-fucosyltransferase that transfers L-fucose to both type 1 and type 2 precursors and thereby provides the H type 1 and H type 2 determinants. The double inheritance of the very rare *h* allele would give rise to either an inactive product or to no product at all, thus explaining the complete lack of H and A or B determinants. In this model, the expression of the *H* gene in salivary glands and erythrocytes would be controlled by two dimorphic regulatory loci: *Se-se* and *Z-z*, respectively [2]. The double inheritance of the recessive alleles *se* and *z* would result in the lack of expression of ABH antigens in saliva and on erythrocytes, respectively.

An analysis of the preferred conformations of type 1 and type 2 precursors suggested the possibility that two different α-2-L-fucosyltransferases may exist [3]. One of the enzymes would use a type 1 precursor as substrate, and the

other, a type 2 precursor. At that time, the type 3 precursor of ABH antigens [4] was not recognized, and the situation may, in fact, be more complex than that stated below. Nevertheless, the possibility of the occurrence of two different H-related enzymes suggested a new genetic model involving only two structural genes coding for two putative fucosyltransferases [5]. Accordingly, the dimorphic *Se-se* locus would be expressed in acinar cells of salivary glands and in some other epithelial cell types of endodermal origin. Since H type 1, H type 2, and H type 3 determinants appear present in the saliva of ABH secretor individuals [4, 6], the fucosyltransferase coded by the *Se* allele could be expected to act on all three precursors. In contrast, the fucosyltransferase encoded by the *H* gene should be expressed on erythrocytes and possibly on some other tissue of mesodermic origin. Since only type 2 ABH determinants have been found on erythrocytes, this latter fucosyltransferase is expected to act only on type 2 acceptors.

Each model allows different predictions concerning the expression of fucosyltransferase activities. According to the classical three-loci model, only one fucosyltransferase should be found, while in the two-loci model, two distinct fucosyltransferases are predicted.

An enzyme with the specificity of an α-2-L-fucosyltransferase has been previously identified in the serum of H-normal individuals [7], and we have recently shown that an α-2-L-fucosyltransferase activity also occurs in the serum of H-deficient secretor individuals [8]. No such activity is found in the serum of H-deficient nonsecretor people [7–9]. Therefore, according to the classical three-loci model, the H-normal nonsecretor and the H-deficient secretor individuals should express the *same* α-2-L-fucosyltransferase coded by the *H* gene but its expression would be blocked in secretion (*H*/-, *se*/*se*, *Z*/-) or in red cells, respectively (*H*/-, *Se*/-, *z*/*z*). In contrast, according to the two-loci model, the fucosyltransferases expressed in the serum of H-normal nonsecretors (*H*/-, *se*/*se*) and the serum of H-deficient secretors (*h*/*h*, *Se*/-) should be different.

We report here in support of the two-loci model for the biosynthesis of H antigenic determinants. A preliminary account of this work has been published recently [10].

MATERIALS AND METHODS

Samples from normal blood group O individuals typed for their Lewis and secretor status were obtained from the Centre National de Transfusion Sanguine, Paris, France. Serum samples from individuals with H-deficient red cells and normal H antigen in saliva (Deu. and Mec.) were obtained through Dr. L. Mannessier, Lille, France, and Dr. L. Rodier, Strasbourg, France. A serum sample from an H-deficient nonsecretor individual, Bombay pheontype (Sel.), used as a reference was obtained from Dr. G. Gérard, la Réunion, France [11]. A very weak A antigenic activity was detected on the erythrocytes of the H-deficient secretor Deu. and a weak H antigenic activity could be detected on the erythrocytes of the H-deficient secretor Mec. [9]. These small A and H antigenic activities could be detected only by the very sensitive adsorption-elution test.

GDP-L-[^{14}C] fucose (190 μCi/μmol) was purchased from NEN Chemicals (Frankfurt, West Germany) and ATP from Sigma (St. Louis, Mo.). Other compounds were: lacto-*N*-Biose I, (βDGal(1→3)DGlcNAc) **2** (Sofichem, Emela-Hayarden, Israel), lactose (βDGal(1→4)DGlc) **5** (Prolabo, Paris, France), phenyl-β-D-galactopyranoside **1** (Koch-

Light, Kolnbrook, U.K.), αL-Fuc(1→6)βD-Gal(1→4)D-GlcNAc **7** and βD-Gal(1→3)D-GalNAc **8** (gift from Prof. P. Sinaÿ, Orléans, France, and Dr. G. Strecker, Lille, France, respectively), βD-Gal(1→3)-4-deoxy-βD-GlcNAc-1-OCH_3 **4**, βD-Gal(1→4)-3-deoxy-βD-GlcNAc-1-OCH_3 **6** (personal communication, D. Khare, O. Hindsgaul, and R. U. Lemieux), and βD-Gal(1→3)βD-GlcNH_2-1 $O(CH_2)_8COOH$ **3** has been reported [12].

α-2-L-Fucosyltransferase Assay

The reaction mixture always contained one of the above-mentioned acceptors, $MgCl_2$ or $MnCl_2$, ATP, 50 mM Tris-HCl buffer, pH 7.2, and GDP-L-[^{14}C]fucose at the concentrations indicated in the legends of figures 1–3 and footnotes to tables 1 and 2, and the solution had a constant final volume of 50 μl. The incubations, unless otherwise stated, were at 37°C and stopped by freezing the samples at −20°C. When **2**, **4**, **5**, **6**, **7**, and **8** were used as acceptors, the residual GDP-L-[^{14}C]fucose was separated from the neutral substances by paper electrophoresis in 0.1 M ammonium formiate buffer, pH 3.5, on Whatman 40, 2,000 V for 2 hrs 30 min. The neutral substances remaining at the origin of the chromatogram were then separated by descending chromatography in pyridine/ethyl-acetate/acetic acid/water (5:5:1:3, v/v) for 8 hrs (solvent A). The [^{14}C]-labeled products were localized on a radiochromatogram scanner (Packard 7201) and quantified by liquid scintillation counting. When **1** and **3** were used as acceptors, descending paper chromatograms were performed immediately after incubation in ethyl-acetate/pyridine/water (10:4:3, v/v) for 6 hrs (solvent B). When lactose **5** was used as an acceptor, the product was eluted and rechromatographed in phenol/2-propanol/formic acid/water (85:5:10:100, v/v) for 40 hrs (solvent C). The reaction products were characterized by their relative mobility taking lactose **5** as a reference.

RESULTS

Optimal Conditions for Enzyme Assay

The fucosyltransferase activity was tested in the serum of an H-normal nonsecretor and of an H-deficient secretor individual, both typed as Lewis positive. Incorporation of L-fucose onto lacto-*N*-Biose I was linear for 15 hrs in serum of the H-normal nonsecretor person and for 5 hrs in serum of the H-deficient secretor individual (fig. 1).

The reaction product was characterized as αL-Fuc(1→2)βD-Gal(1→3)-D-GlcNAc as previously deduced from chromatographic mobility compared to an authentic trisaccharide and degradation by a specific fucosidase [13]. In both types of sera, the α-2-L-fucosyltransferase activity was optimum at pH 7.2. Also in both sera, addition of 20 mM $MnCl_2$ or 10 mM $MgCl_2$ maximally enhanced the enzymatic activity. It was noted in other experiments that the enzyme activities in both sera were only doubled upon addition of 10 mM $MgCl_2$.

ATP was added in order to prevent an excessive degradation of GDP-fucose. Maximal α-2-L-fucosyltransferase activity was obtained at a final concentration of 5 mM in both sera tested. At this concentration, less than 1% of [^{14}C]fucose was released from GDP-L-[^{14}C]fucose after 5-hrs incubation at 37°C.

GDP-L-[^{14}C]fucose was saturating at 0.04 mM in the H-normal nonsecretor serum, and 0.09 mM was considered as close enough to saturation in the H-deficient secretor serum.

Using optimum conditions for pH, Mg^{2+}, and ATP, saturating GDP-fucose, and 5-hr incubation time at 37°C, the α-2-L-fucosyltransferase activities of the

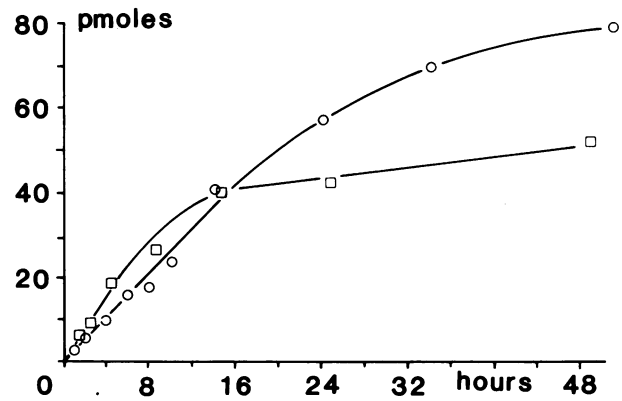

FIG. 1.—Time course for the incorporation of [^{14}C]fucose into lacto-*N*-Biose I catalyzed by the serum from an H-deficient secretor person Deu. (□) and the serum from an H-normal nonsecretor donor (○). The assay conditions are those presented in table 1 using 4.7 mM of lacto-*N*-Biose I, and, in the case of GDP-L-[^{14}C]fucose, 0.012 mM for the H-normal nonsecretor serum (○) and 0.048 mM for the H-deficient secretor (□). *Ordinate*: pmol of [^{14}C]fucose transferred onto lacto-*N*-Biose I quantified as described in MATERIALS AND METHODS.

H-normal nonsecretor and of the H-deficient secretor sera were compared using lacto-*N*-Biose I **2** as acceptor. Saturation was reached for both sera at 4.7 mM of **2**, and the amounts of [^{14}C]fucose incorporated were nearly the same in both sera.

Substrate Specificity and Kinetic Analysis

The apparent Michaelis constant (K_m) and maximum velocity (V_{max}) of the fucosyltransferases were determined for GDP-fucose at saturating concentration (9.4 mM) of lacto-*N*-Biose I **2**. Figure 2 shows that the apparent K_m for GDP-fucose was different in both sera. The values were 0.008 mM and 0.028 mM for H-normal nonsecretor and for H-deficient secretor sera, respectively. The apparent V_{max} for GDP-fucose were similar in both sera: 290 pmol/hr per ml and 280 pmol/hr per ml, respectively. These results suggested that the α-2-L-fucosyltransferase of the H-deficient secretor serum had a lower affinity for GDP-fucose than did the α-2-L-fucosyltransferase of the H-normal nonsecretor serum but that the transfer of fucose onto lacto-*N*-Biose I **2** occurred at similar rates in both sera.

The apparent K_m and V_{max} for other acceptors are presented in table 1. Using the H-deficient secretor serum as source of fucosyltransferase, the apparent K_m for lacto-*N*-Biose I **2** and lactose **5** were identical (1 mM), but the apparent V_{max} differed largely (170 and 40 pmol/hr per ml, respectively). It appeared that the [^{14}C]fucose incorporated in lactose was linked α(1→2) to the galactosyl residue, since, upon rechromatography in solvent C, the enzymic product was characterized by an Rlac value of 1.0 [14]. Certainly, this result indicates that the enzyme present in the H-deficient secretor serum recognized both substrates but that the transfer of fucose to lactose **5** was much slower than to lacto-*N*-Biose I **2**.

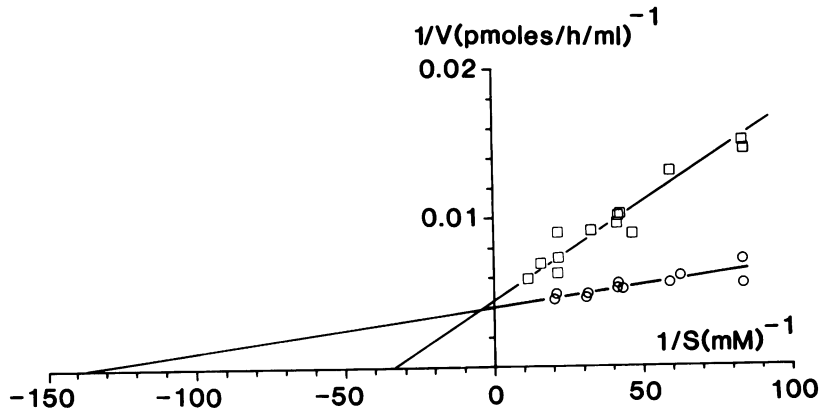

FIG. 2.—Lineweaver and Burk plots for determination of apparent K_m and V_{max} values of α-2-L-fucosyltransferases in the serum from an H-deficient secretor individual (□) and an H-normal nonsecretor individual (○). The assay conditions were the same as those described in table 1 using 9.4 mM of lacto-*N*-Biose I.

In contrast, the enzyme present in the H-normal nonsecretor serum did not differentiate between the two acceptors. Both the apparent K_m and V_{max} values for these two acceptors were identical.

When de-*N*-acetylated type 1 precursor βDGal(1→3)βDGlcNH$_2$-O-(CH$_2$)$_8$-COOH **3** was used as an acceptor for the fucosyltransferase from the H-deficient secretor serum, both apparent K_m and V_{max} values (table 1) were quite similar to those obtained for the *N*-acetylated type 1 precursor **2**. In contrast, apparent V_{max} for the enzyme present in the H-normal nonsecretor serum using **3** as acceptor was only about one-half that measured for **2** (135 pmol/hr per ml) and 250 pmol/hr per ml, respectively) (table 1).

αLFuc(1→6)βDGal(1→4)DGlcNAc **7** was a poor acceptor for the α-2-L-fucosyltransferase in the serum of the H-deficient secretor as compared to the type 1 structures **2** and **3**. The apparent K_m was four times less, and the V_{max} value was 1.7 times greater. In contrast, using the serum of the H-normal nonsecretor, the apparent K_m for **7** was less, and the V_{max} was greater than for **2** (table 1).

For phenyl β-D-galactopyranoside **1** as acceptor, the apparent K_m was 10 times higher than for **2** using the serum of the H-deficient secretor. On the other hand, using the serum of the H-normal nonsecretor, the apparent K_m for **1** was less than that for **2**. Also, the V_{max} values in this serum were greater for **1** than for **2**.

Relative Rates of Incorporation on Various Sugar Acceptors

The extent of incorporation of [^{14}C]fucose onto a series of acceptors was determined using saturating conditions for GDP-fucose (0.045 mM and 0.09 mM for H-normal nonsecretor and H-deficient secretor sera, respectively) and in each case, 0.5, μmol of the acceptor per 50 μl (final solution). The results are

TABLE 1

SUBSTRATE SPECIFICITIES OF α-2-L-FUCOSYLTRANSFERASES IN HUMAN SERA

SUGAR ACCEPTOR	RANGE OF CONCENTRATION	RLac†	SERUM ENZYMES* H-NORMAL NONSECRETOR K_m (mM)	H-NORMAL NONSECRETOR V_{max} (pmol/hr/ml)	H-DEFICIENT SECRETOR‡ K_m (mM)	H-DEFICIENT SECRETOR‡ V_{max} pmol/hr/ml
Phenyl-β-D-galactopyranoside **1**	0.4–3.2	4.0 (B)	1.4	330	10.0	250
Type 1 acceptors:						
βDGal(1 → 3) DGlcNAc **2** (lacto-*N*-Biose I)	0.3–4.7	1.1 (A)	2	250	1	170
βDGal(1 → 3) βDGlcNH$_2$-O(CH$_2$)$_8$COOH **3**	0.9–3.6	1.9 (B)	1.9	135	1.3	170
Type 2 acceptors:						
βDGal(1 → 4)DGlc **5** (lactose)	0.3–5.2	0.9 (C)	2	250	1	40
αLFuc(1 → 6)βDGal(1 → 4) DGlcNAc **7**	0.6–2.4	1.0 (A)	1.3	450	4.5	100

* The assays were performed using serum (25 μl), 50 mM Tris (hydroxymethyl) aminomethane-HCl (5 μl, pH 7.2), $MgCl_2$ (0.5 μmol), ATP (0.25 μmol), GDP-L[^{14}C]fucose (2.3 nmol for H-normal nonsecretor serum and 4.6 nmol for H-deficient secretor serum) and acceptor in the range indicated prior to dilution to a final volume of 50 μl. After incubation for 5 hrs at 37°C, the apparent K_m and V_{max} values were determined as indicated in the text.

† Relative paper chromatographic mobility using the solvent system indicated in parentheses (see MATERIALS AND METHODS).

‡ No transfer of L-fucose was catalyzed by the serum of an H-deficient nonsecretor using **2** as acceptor.

reported in table 2 using the radioactivity introduced into lacto-*N*-Biose I **2** as representing 100% incorporation.

The acceptors lacto-*N*-Biose I **2** and lactose **5** were assayed with four sera from unrelated H-normal nonsecretors and two sera from unrelated H-deficient secretors. Table 2 shows that the four H-normal nonsecretor sera gave highly reproducible results with these two acceptors (footnote *). One of these H-normal nonsecretor sera was tested with the whole series of selected acceptors **1–8**, and it showed high transfer efficiency to all the subtrates except the type 3 dissacharide **8**, which was a relatively poor acceptor.

The fucosyltransferase present in the sera from H-deficient secretor persons showed a marked preference for the type 1 **2–4** and type 3 **8** subtrates. The type 2 compound **6** did not even function at all as an acceptor under the experimental conditions.

Heat Inactivation of Fucosyltransferases

Preincubation of sera at 46°C for various lengths of time were performed as described previously for A and B glycosyltransferases [15]. Figure 3 shows that the α-2-L-fucosyltransferase activity present in the H-deficient secretor serum decreased much faster than the activity present in the H-normal nonsecretor

TABLE 2

RELATIVE RATES OF TRANSFER OF L-FUCOSE TO DIFFERENT OLIGOSACCHARIDE ACCEPTORS BY α-2-L-FUCOSYLTRANSFERASES IN HUMAN SERA

SUGAR ACCEPTORS	H-NORMAL NONSECRETOR SERUM*	H-DEFICIENT SECRETOR SERA	
		Deu.	Mec.
Phenyl-β-D-galactopyranoside **1**	164	28	n.d.†
Type 1 acceptors:			
βDGal(1→3)DGlcNAc **2** (lacto-*N*-Biose I)	100	100	100
βDGal(1→3)βDGlcNH$_2$-1 O(CH$_2$)$_8$COOH **3**	47	79	n.d.
βDGal(1→3)-4-deoxy-βDGlcNAc-1 OCH$_3$ **4**	55	75	n.d.
Type 2 acceptors:			
βDGal(1→4)DGlc **5** (lactose)	100	22	30
βDGal(1→4)-3-deoxy-β DGlcNAc-1 OCH$_3$ **6**	78	0	n.d.
αLFuc(1→6)βDGal(1→4)DGlcNAc **7**	220	20	n.d.
Type 3 acceptor:			
βDGal(1→3)DGalNAc **8**	29	n.d.	116

NOTE: The assays were as described in table 1 using 10 mM of the acceptor. In the case of the H-deficient secretor sera, 0.09 mM of GDP-L[^{14}C]fucose was used.

* Sera from three other unrelated H-normal nonsecretor individuals were tested using lacto-*N*-Biose I **2** and lactose **5** as acceptor substrates. Taking the incorporations onto lacto-*N*-Biose I as 100%, the incorporations onto lactose were 89%, 98%, and 100%, respectively.

† n.d. = not determined.

serum. The half-times of inactivation were 10 min and 75 min, respectively, indicating large differences in stability. This result is consistent with the fact that fucosylations at 37°C were linear for 5 hrs and 15 hrs, respectively (fig. 1).

DISCUSSION

Two types of sera were compared for their β-D-*Gal* α-2-L-fucosyltransferase activities: (1) sera from H-normal nonsecretors (*H/-*, *se/se*) and (2) sera from H-deficient secretors (*h/h*, *Se/-*). The acceptors **1–8** used in this study were carefully chosen so as to avoid competition with the β-D-*GlcNAc* α-3-L-

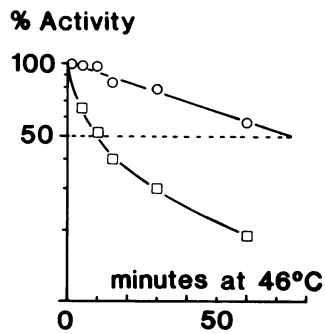

FIG. 3.—Heat-denaturation of α-2-L-fucosyltransferases in serum from an H-deficient secretor person (□) and an H-normal nonsecretor person (○). Prior to each assay, the sera were incubated at 46°C for the periods of time shown on the *abcissa*. The assay conditions were the same as those reported in table 1 but using 0.024 mM of GDP-L-[^{14}C]fucose and an incubation time of 22 hrs at 37°C.

fucosyltransferase present in the serum of all individuals. Indeed, no incorporation of [^{14}C]fucose was evidenced to any of these acceptors using the serum of an H-deficient nonsecretor (*h/h*, *se/se*) as source of enzyme. This serum contained a strong β-D-*GlcNAc* α-3-L-fucosyltransferase as estimated by the specific fucose incorporation of [^{14}C]fucose to *N*-acetyllactosamine (data not shown). In addition, competition with the β-D-*GlcNAc* α-4-L-fucosyltransferase under control of the Lewis gene could not occur since this last enzyme is not present in serum [1].

Three sets of results are indicative that the β-D-*Gal* α-2-L-fucosyltransferase present in the serum of the H-normal nonsecretors is different to that present in the serum of the H-deficient secretors. (1) The apparent K_m for GDP-fucose was four times higher for the enzyme present in the serum of an H-deficient secretor individual than for the enzyme present in the serum of an H-normal nonsecretor individual. (2) Type 1 acceptors **2–4** and type 3 acceptor **8** were good acceptors in the serum of H-deficient secretor individuals (*h/h*, *Se/-*), whereas type 2 acceptors **5–7** and phenyl-β-D-galactopyranoside **1** were very poor acceptors. On the other hand, as was to be expected from the high abundance of H type 2 determinants on the red cells of H-normal individuals, the fucosylation of the type 2 structures **5**, **6**, and **7** was much more extensive relative to **2** in the serum of the H-normal nonsecretor (*H*, *se/se*). Phenyl-βD-galactopyranoside **1** was also a good acceptor in this serum, whereas the type 3 compound **8** was the poorest acceptor. (3) Further differences between the β-D-*Gal* α-2-L-fucosyltransferases present in the two sera were obtained following heat-denaturation experiments. The fucosyltransferase activity from the H-deficient secretor serum was more susceptible to heat inactivation than was the enzyme from the H-normal nonsecretor serum. In addition, as estimated from the shape of the denaturation curves, it appears that the denaturation processes follow different order kinetics in the two sera.

Serum samples from H-normal secretor individuals (*H/-*, *Se/-*) were also studied (data not shown). In these sera, a mixture of the two fucosyltransferases was expected. In fact, only one enzymatic activity with the properties of the α-2-L-fucosyltransferase present in H-normal nonsecretor sera could be demonstrated. This can be explained by the fact that, in these experiments, even 0.09 mM GDP-fucose was not saturating for the two enzymes. Since the enzyme under control of the *Se* gene has a K_m for GDP-fucose four times higher than the enzyme under control of the *H* gene, only this latter enzyme was detectable. The failure of previous workers [16, 17] to identify β-D-*Gal* α-2-L-fucosyltransferase activity in the serum of H-deficient secretor individuals using **1** as acceptor may be related to the relatively high apparent K_m and the use of low concentrations of either GDP-fucose or **1**.

In conclusion, we wish to point out a number of previous observations that are in accord with the data presented above. Chester et al. [18] reported that the βD-*Gal* α-2-L-fucosyltransferase in H-normal sera transferred fucose equally well to lacto-*N*-Biose I and lactose but that phenyl-β-D-galactopyranoside was a superior acceptor. Scudder and Chantler [19] observed that lacto-*N*-Biose I was a better acceptor than either lactose or phenyl-β-D-galactopyra-

noside for a human cervical epithelium β-D-*Gal* α-2-L-fucosyltransferase. Moreover, Clamagirand-Mulet et al. [20] found that the β-D-*Gal* α-2-L-fucosyltransferase present in human milk and, therefore, under the control of the *Se* gene, did not detect phenyl-β-D-galactopyranoside as an acceptor and detected lactose only very weakly. It has been recently shown that the α-2-L-fucosyltransferase from the submaxillary gland of a secretor person favored type 1 to type 2 acceptors, whereas the plasma enzyme from an H-normal person did not distinguish between the two types of acceptors [21]. More recently, Kumazaki and Yoshida [22] compared the properties of the α-2-L-fucosyltransferase from H-normal sera and that of milk (Se). These authors reported differences in heat stability and kinetic properties between the enzymes from the two sources also in accordance with the results presented herein. Indeed, the plasma enzyme under control of *H* gene was more heat-stable than that from milk under control of the *Se* gene. Furthermore, it was shown that the enzyme under control of the *H* gene favored phenyl-β-D-galactopyranoside as an acceptor but did not distinguish type 1 and type 2 substrates and, most importantly, that the enzyme under control of the *Se* gene strongly favored the type 1 acceptor. This shows that the α-2-L-fucosyltransferase under control of the *Se* gene exhibits similar properties whether it originates from salivary glands [21], milk [22], or serum (this study). It has been suggested that the β-D-*Gal* α-2-L-fucosyltransferases present in sera originate from hematopoietic tissues [16, 17]. The present results strongly suggest that these are under the control of the *H* gene and, in the case of secretors, from the epithelial compartment under the control of the *Se* gene that contributes a different β-D-*Gal* α-2-L-fucosyltransferase to the pool. The difference between the two enzymes could arise by post-translational modifications of the product of the H gene. This hypothesis would require post-translational modifications that provide one isoenzyme to act preferentially on type 1 substrates in tissues of endodermal origin and a second isoenzyme in tissues of mesodermal origin with a preference for type 2 acceptors. Alternatively, the difference between the two enzymes can be explained by the existence of two structural genes, the *H* and *Se* genes, as was earlier proposed [3, 5].

The *H* and *Se* genes are closely linked [5], and ABH antigens have been found in secretions from humans and all other mammals. For example, Beyer and Hill [23] found that lacto-*N*-Biose I was a better acceptor than lactose for a purified β-D-*Gal* α-2-L-fucosyltransferase from porcine submaxillary glands as was found in this present investigation for the enzyme in the sera of the H-deficient secretors. On the other hand, the type 2 ABH antigens of red cells have been found only in man and some anthropoid apes [24]. As was previously proposed [6], the *H* gene could have originated from a duplication of the ancestral *Se* gene.

The list of proteins known to be encoded by multigene families with developmentally regulated expression has considerably grown in recent years. The human β-D-*Gal* α-2-L-fucosyltransferases may be another example of such proteins.

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THE INTERNATIONAL SYMPOSIUM OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE will be held November 14–16, 1985, in Pasadena, California. Sponsored by the National Institutes of Health and the City of Hope National Medical Center, the topics will include: G6PD deficiency and hemolytic anemia, G6PD activity and red cell metabolism, G6PD variations, G6PD in non-human organisms, expression of Gd locus, G6PD structure and function, and cloning and structure of the gene. Further information is available from: A. Yoshida, Department of Biochemical Genetics, City of Hope National Medical Center, Duarte, CA 91010. Telephone: (818)359-8111, ext. 2362.